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“Rho”ing a cellular boat with rearward membrane flow

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Abstract

The physicist Edward Purcell wrote in 1977 about mechanisms that cells could use to propel themselves in a low Reynolds number environment. Reporting in *Developmental Cell*, O'Neill et al. (O'Neill, 2018) provide direct evidence for one of these mechanisms by optogenetically driving migration of cells suspended in liquid through RhoA activation.

Main text

The need for self-propulsion is a fundamental challenge facing cells across diverse forms of life and cellular environments. Not surprisingly, evolution has found multiple solutions to this problem, with migration modes varying from flagella-based swimming to adhesive crawling along surfaces. For migrating eukaryotic cells, the best characterized modes of migration rely on the formation of actin-based protrusions that push the cell's leading edge forward, while adhesion complexes provide traction against an extracellular surface or matrix. In this context, myosin-based contraction is often described as pulling the back of the cell forward, following the leading role of protrusion at the front. More recently, however, alternative modes of cell motility were characterized, for which classical integrin-mediated adhesion is not required (Paluch et al., 2016). Furthermore, neutrophils and *Dictyostelium discoideum* amoeba, classic models of crawling cells, can also propel themselves by "swimming" when suspended in liquid (Barry and Bretscher 2010). Reporting in *Developmental Cell*, O'Neill and colleagues use optogenetic approaches to drive different modes of cell migration including cell "swimming". These results shed light onto the basis of these less explored migration mechanisms, and provide a different perspective on the role of the cell rear in motility.

For cells to effectively "swim", they must contend with the unique challenges of their liquid surroundings. At their size scale, cells encounter a low Reynolds number environment: inertial forces are negligible relative to much larger viscous forces. A consequence is that cells cannot coast or glide. Instead, persistent cellular movement requires a cycle of shape changes that interact with the surrounding fluid in a non-reciprocal way. Physicist Edward Purcell described this phenomena in his "Scallop Theorem", which states that if a sequence of movements are time reversible, like the opening and closing of a scallop shell, then the organism will fail to propel itself (Purcell, 1977). Similarly, he noted that it would be impossible to row a boat using rigid oars under these conditions. In his seminal work, Purcell also proposed a number of theoretical swimming modes that could function at low Reynolds number. One example was the "toroidal swimmer", where a donut-shaped cell could "swim" by directionally treadmilling its plasma membrane (Figure 1A). A later theoretical study calculated the maximum speed for a spherical cell using an analogous swimming mode as ~ ⅔ of the membrane treadmilling rate (Leshansky and Kenneth, 2007). Excitingly, O'Neill et al. (2018) now show that surface treadmilling controlled by active RhoA at the cell rear (Figure 1B) is sufficient to drive directional cellular motility on 2D surfaces and in liquid using a mechanism that is reminiscent of Purcell's toroidal swimmer at a rate similar to that computed by Leshansky and Kenneth.

However, the authors began by asking a more basic question about which pathways are sufficient to drive cell motility. In many directed cell migration pathways, external stimuli are sensed by G-Protein Coupled Receptors (GPCRs). These receptors activate a network of downstream effectors, in which the Rho-family GTPases Rac and Cdc42 are primary organizers of the cell front, and RhoA is a primary regulator of the rear. Local activation of either Rac or Cdc42 is sufficient to drive forward cell migration (O'Neill et al., 2016; Wu et al., 2009). The authors asked whether polarized RhoA activation and the

resulting contractile actomyosin complexes would also be sufficient to drive cell motility from the rear. Indeed, not only was optogenetically-activated RhoA (Figure 1C) sufficient to trigger cell migration, but it also produced a motility mode that differed markedly from that induced by activation of optogenetic GPCRs (Figure 1D) at the cell front! While GPCR-driven migration is characterized by a ruffling leading edge with dynamic actin-based protrusions induced by the signaling activities of Rac and Cdc42, RhoA-driven cells had little protrusive activity at the front and were unaffected by a chemical Rac inhibitor. Even so, they moved with similar speed.

If these cells don't have a protrusive front, how are they propelling themselves? By imaging membrane associated proteins, the authors show that these cells generate persistent rearward plasma membrane flow that converges on the region of RhoA activation. To recycle accumulating membrane from the cell rear to the front, cells trigger rear-associated endocytosis followed by anterograde vesicle transport. Importantly, both the membrane flow and migration required endocytosis and vesicular trafficking. Based on these observations, the authors proposed that the RhoA-driven cells are treadmilling their surface rearwards, through a polarized circuit of membrane secretion at the cell front and endocytosis at the rear, with RhoA-directed myosin-based contraction generating a rearward cortical cytoskeletal flow that carries the other membrane components with it (Figure 1B). To test this model, they verified that the activity of the kinase ROCK, which acts downstream of RhoA to activate myosin, is required.

To address whether RhoA-driven migration can function in a truly adhesion-independent manner, the authors performed a cell "swimming" experiment by locally activating RhoA in cells that were suspended in a solution of matching density – with no surface or 3D matrix present for the cells to interact with. Again, they compared GPCR- and RhoA-driven cells. Surprisingly, while the GPCR-stimulated cells did generate a ruffling front, they failed to move or generate any membrane treadmilling. In contrast, the RhoA-driven cells used membrane treadmilling to move just as fast as they did on a surface. We note that the GPCR result reported here differs from the GPCR-driven chemotactic swimming observed for neutrophils and *Dictyostelium* amoeba (Barry and Bretscher, 2010). This disparity may reflect differences in the wiring of the motility machinery; faster moving cells may generate more rear contractile activity downstream of GPCR inputs.

Interestingly, a recent study in *Dictyostelium* amoeba analyzed membrane flow during migration through a compressed space (Tanaka et al., 2017). By using FRAP to analyze the diffusion and flow of a fluorescently-labeled lipid, they found that both the apical and basal membranes of the cell flowed backwards relative to the cell's leading edge at a speed that matched the speed of cell migration. This suggests that similar membrane flow and polarized vesicular transport may be a general feature of a broader family of cell migration modes, including those thought to be driven primarily by actin-based protrusion at the front.

In summary, the new report by O'Neill and colleagues (2018) provides insights into the basic mechanisms that eukaryotic cells use to propel themselves, and into the wiring of signaling networks that control cell migration. Excitingly, their discovery of the Opto-RhoA swimmer finally provides experimental validation of the surface flow model for low Reynolds number swimming postulated just over thirty years ago in Purcell's "toroidal swimmer" theory. Additionally, their results suggest that rearward membrane flow may contribute to cell propulsion in many contexts, including the interstitial migration of immune cells, and adhesion-independent modes of migration seen in microchannels and confined environments in culture (Paluch et al., 2016). To understand its broader relevance, it will be

important to determine how the polarized vesicular trafficking is established, and whether the same mechanisms occur in other modes of cell migration.

Figure 1: Cell swimming models and optogenetic stimulation tools. A) Diagram of Purcell's theoretical toroidal swimmer. Membrane flow around the toroidal shape drives forward cell displacement. B) Diagram of the Opto-RhoA swimmer. Local activation of RhoA (orange gradient) triggers rearward membrane treadmilling (arrows). This membrane flow drives forward cell movement. However, unlike the torus, the membrane must be recycled to the cell front by anterograde vesicular trafficking. C) Schematic for optogenetic RhoA activation using the iLid system. Blue light stimulation triggers the membrane anchored iLid domain to expose the SsrA peptide (yellow), recruiting the SspB-LARG fusion protein to the membrane, where LARG activates RhoA. Opto-RhoA generates a rounded cell shape with a small uropod in the area of highest RhoA activity. D) Schematic for optogenetic GPCR activation. Here, blue light activates a light-sensitive GPCR to trigger a chemotaxis-like response. GPCR activity builds a ruffling leading edge that the cells use for adherent crawling. This method did not trigger uropod formation.

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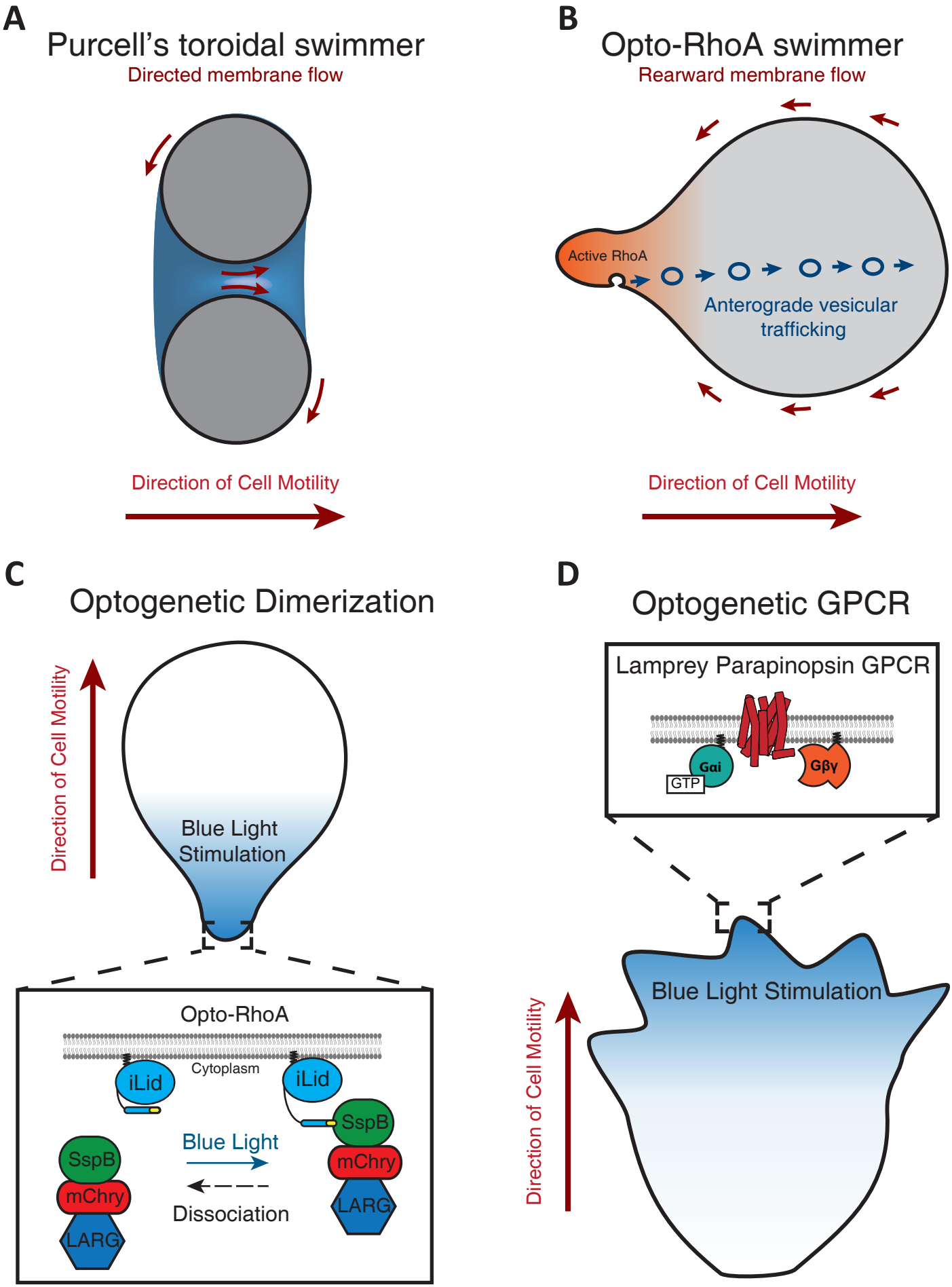
A
Purcell's toroidal swimmer
Directed membrane flow
Direction of Cell Motility
B
Opto-RhoA swimmer
Rearward membrane flow
Active RhoA
Anterograde vesicular trafficking
Direction of Cell Motility
C
Optogenetic Dimerization
Direction of Cell Motility
Blue Light Stimulation
Opto-RhoA
Cytoplasm
iLid
iLid
SspB
mChry
LARG
Blue Light
Dissociation
SspB
mChry
LARG
D
Optogenetic GPCR
Lamprey Parapinopsin GPCR
Gαi
GTP
Gβγ
Blue Light Stimulation
Direction of Cell Motility